FINAL REPORT:

Can on-site beef dark cutting evaluation (monitoring) be improved and value-added?

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1.0 EXECUTIVE SUMMARY

Colour contributes to consumer perceptions of beef quality and value, and therefore any beef with unacceptable colour is often discounted by industry. Dark cutting (DC) is characterised by its dark, firm and dry characteristics and its incidence is a concern to industry.

Conventional carcass grading (assessment) will categorise a beef carcass as DC or otherwise (nDC) based on the measurement of the loin – which acts as a single 'marker muscle'. This grade and it's associated reduced value is then applied to the whole carcass.

In Australia, it is common for beef carcasses to be graded within the first 24 h *post-mortem*. This often occurs between 12-16 h when trained abattoir staff insert pH probes into the loin and grade the carcass as DC if pH > 5.7.

We aimed to ascertain within-carcass quality trait variation for DC and nDC beef; understand rate of glycolysis and pH declines over the first 24 h *post-mortem*; compare NIX Pro Color Sensor™ colorimetrics potential to distinguish between DC and nDC beef carcasses; and, explore the relationship between these colour measures and other meat quality traits indicative of shelf-life and eating quality.

DC effects on tenderness traits were not uniform across all cuts, with the bolar blade exhibiting no difference in DC and nDC carcasses for colour traits. Only lipid oxidation (measured as TBARS) was influenced by grade and ageing period interactions. Colorimetric variation due to grade was more evident in the striploin than the other cuts, although this was independent to ageing or display. Cuts themselves differed and were impacted by ageing and display periods.

Therefore, it is reasonable to conclude that at least the bolar blade and potentially the forequarter of beef carcasses classified as DC do not reflect the negative attributes of the striploin and topside, and could therefore retain the value of nDC counterparts – particularly carcasses classified as 'slightly DC' as used in the present study.

Modelling pH and glycolytic metabolites (glucose, glycogen, lactate, glucose-6-phosphate and glycolytic potential) demonstrated a difference between the three cuts evaluated in their rates of glycolysis, but a shared continuation or metabolic instability within the first 24 h *post-mortem*. This suggests that conventional grading (12-16 h *post-mortem*) may be premature and misrepresent the final status of a beef carcass.

We observed that NIX colorimetrics (L*, a*, b*, hue and chroma values) offer an objective means to differentiate between DC and nDC beef carcasses at grading and as per Australian industry practice. Specifically, a chroma threshold at 30.5 provides the highest total specificity and sensitivity when applied to this task.

The bolar blade, striploin and topside from a selection of DC and nDC carcasses were also removed, aged for 14 d and tested for shear force, drip loss, cooking loss, ultimate pH, colour change over 3 d display, and sarcomere length. Association between chroma values and these quality traits were investigated by linear models. But, there was only limited usefulness in predicting other meat quality traits with the recorded NIX colorimetrics for the bolar blade, striploin and topside cuts.

2.0 INTRODUCTION

Dark cutting (DC) is problematic to the Australian beef industry (McGilchrist, Perovic, Gardner, Pethick, & Jose, 2014) and in an effort to discourage its prevalence; processors will discount and downgrade the value of these carcasses. This action is based on the consumers' preference for beef with a bright red appearance (Mancini & Hunt, 2005) and as DC beef fails to match this criterion it is instead considered less fresh and of lower quality than normal or non-DC (nDC) beef (Forbes, Vaisey, Diamant, & Cliplef, 1974). This fundamental difference is cited to result from DC carcasses having insufficient glycogen reserves to drive *post-mortem* acidification (Honikel, 2014). Furthermore, this atypical glycogen-pH paradigm can detract from beef water holding capacity and eating quality traits, and its susceptibility to oxidation. As a consequence, industry often uses colour and/or pH characteristics to objectively identify DC beef.

It is normal for beef carcass appraisal and categorisation as DC or nDC (grading) to occur within the first 24 h post-mortem and involves a trained operator judging an exposed loin surface against a colour and/or pH standard reference. Ponnampalam et al. (2017) reviewed the variation in grading references between markets, countries and research projects. Albeit, for the purpose of this study, we adhered to the Meat Standards Australia recommendation of pH > 5.7 as being indicative of DC (MLA, 2017). That said, common to the majority of grading practices are their use of a single 'marker muscle' (the loin) to classify the entire carcass. This observation is noteworthy as past research has reported within-carcass glycolytic and pH differences. For example, at 40 min *post-mortem* Przybylski, Vernin, & Monin (1993) found beef *m. longissimus lumborum*, *m. psoas major* and *m. rectus abdominis* each had different pH values and glycolytic potentials. Talmant & Monin (1986) compared 18 different muscles, sampled at 30 min *post-mortem*, to also find glycolytic potential differences, but also ATPase activity and ultimate pH values that reflected this variation – including between the *m. longissimus lumborum*, *m. semimembranosus* and *m. triceps brachii*. Furthermore, Bendall (1977) identified a variation between several muscles in their rate of pH decline *post-mortem*. Together, these differences in pH and glycolytic traits are likely due to differences in glycogen reserve distributions around the carcass (Bass et al., 2008).

Based on these observations, grading practices may be misrepresenting the prevalence or expression of DC traits in beef cuts other than the striploin. In response, we aimed to ascertain within-carcass quality trait variation for DC and nDC beef. This was realised by comparing the eating quality, colour stability and water holding capacity traits of aged bolar blade, striploin and topside beef cuts removed from DC and nDC carcasses.

The rate of post-mortem glycolysis can influence two central mechanisms that profoundly impact myofibrillar tenderness *viz.* the extent of myofibrillar contraction and the degree and rate of proteolysis during ageing (Ferguson & Gerrard, 2014). Different fibre-type profiles of muscles within the same carcass drive this glycolysis, with fast-twitch and glycolytic having greater glycolytic potential than slow-twitch and oxidative muscles (Bouton, Carrol, Harris, & Shorthose, 1973; Kirchofer, Calkins, & Gwartney, 2002; Klont, Brocks, & Eikelenboom, 1998). Consequently, understanding the post-mortem time point at which key glycolytic metabolites stabilise and whether this varies within-carcass could be valuable to grading schedules that better capture beef carcass status. Consequently, we aimed to monitor pH and glycolytic metabolite variation across the first 24 h post-mortem in the bolar blade, striploin and topside of individual beef carcasses.

The use of subjective measurements when grading carcasses can be problematic (Hulsegge, Engel, Buist, Merkus, & Klont, 2001), especially when justifying carcass discount or rejection, and therefore pH has been tasked as an objective measurement to define DC (MLA, 2017). Objective colour measurements in this same application have proven more challenging due to their cost, complexity and suitability to operate within abattoir conditions. The Nix Pro Color Sensor™ (NIX) has emerged as an alternative to conventional colorimetric instruments because of its relative precision, cheapness and user-friendly interface (Holman & Hopkins, 2019; Nix Sensor Ltd., 2018). Nonetheless, and to the best of our knowledge, its application to the grading of beef carcasses remains to be confirmed.

Beef quality traits other than DC are impacted by the same post-mortem glycogen-pH interactions. These include eating quality, colour stability and water-holding characteristics (Ponnampalam et al., 2017; Puolanne & Immonen, 2014; Wu, Farouk, Clerens, & Rosenvold, 2014). Understanding the relationship between colorimetric measures made when grading beef carcasses to these traits could therefore offer an opportunity for industry to value-add to the information gleaned from an otherwise routine inspection. However, as only the loin is assessed when a carcass is graded (MLA, 2017), it is important that its representation of other cuts is also established. As a result, we compared NIX colorimetric capacities to distinguish between DC and nDC beef carcasses and explored the relationship between these colour measures and other meat quality traits indicative of shelf-life and eating quality.

3.0 PROJECT OBJECTIVES

This project aimed to:

- // Determine DC parameter variation between beef muscles (with-carcass) and formulate a monitoring guide (when and where) to improve evaluation precision, accuracy and whole carcass representation.
- // Evaluate the capacity for monitored DC parameters to provide additional information to industry in terms of product shelf-life, spoilage, and purge characteristics.

4.0 METHODOLOGY

4.1 Experiment One

4.1.1 Sample collection

From the chiller of a commercial Australian abattoir, a total of 30 grass-fed beef carcasses graded as DC (n = 16) or nDC (n = 14) were selected (Table 4.1). Grades were assigned at ~ 12-16 h *post-mortem* and in accordance to normal industry practice (MLA, 2017) – wherein the striploin HAM: 2140 (Anonymous, 2005); *M. longissimus lumborum*) surface between the 12-13th rib was exposed, allowed to bloom for ~ 1.5 h at 2-3 °C, and evaluated so that when its pH was > 5.7, the carcass was considered as DC. It was from these experimental carcasses that the striploin, bolar blade (HAM: 2302 (Anonymous, 2005); *M. triceps brachii*) and topside (HAM: 2001 (Anonymous, 2005); *M. semimembranosus*) were removed. All cuts were then vacuum-packaged and aged for 14 d on-site and again, in accordance with normal industry practice. These were then transported to the Centre for Red Meat and Sheep Development (Cowra, AUS) where they were portioned to be aged for an additional 14 d (so that there were two ageing periods; 14 d and 28 d) or immediately sectioned for

quality trait determination. These latter portions were held at – 25 °C until their analysis – with colour stability sample slices and drip loss cores the exception, as these were assessed 'unfrozen'.

Table 4.1

Summary data for the experimental beef carcasses graded (by industry) as dark cutting (DC) or control (nDC). Averages are shown with minimum and maximum values included in parenthesis.

Parameter	DC	nDC
Number	16	14
Sex	6 (female) 10 (male)	10 (female), 4 (male)
Hot carcass weight	149.0 kg (117.3-202.9 kg)	145.2 kg (126.1-167.9 kg)
pH (at grading)[1]	5.96 U (5.44-6.43 U)	5.48 U (5.26-6.32 U)
Temperature (at grading)[1]	9.4 °C (7.5-11.2 °C)	9.7 °C (8.1-11.4 °C)

[1]Measured on the exposed striploin surface between the 12-13th rib in-plant, at grading

4.1.2 Drip loss

Drip loss (DL) was determined using fresh sample cores (diameter: 2.5 cm), which were weighed prior to being placed in EZ-DripLoss tubes (Danish Meat Research Institute, Taastrup, DEN). These were held under refrigeration for 72 h before cores were reweighed, and DL was calculated as a samples percentage weight change over this period.

4.1.3 Cooking loss and shear force

Vacuum-packaged and frozen sample shear force (SF) blocks (mean ± standard deviation: 68.0 ± 5.2 g) were submerged in a 71°C water bath (90 L with a 2000 W heating element, Ratek Instruments Ltd., Melbourne, AUS) for 35 min, then removed and held under cold tap water for 30 min – to halt the cooking process (Hopkins, Toohey, Warner, Kerr, & van de Ven, 2010). Packaging was then removed, samples were patted dry and then reweighed so that cooking loss (CL) could be calculated as the percentage weight change pre- and post-cooking. These where then refrigerated overnight (at 3-4 °C). Six cuboidal strips (cross-sectional area: 1 cm^2) were then removed from each sample block, parallel to the muscle fibre orientation (Holman, Alvarenga, van de Ven, & Hopkins, 2015). A texture analyser (Model LRX, Lloyd Instruments, Hampshire, UK) with attached Warner-Bratzler blade set to 200 mm crosshead speed was used to measure the peak force (Newtons) to cut each strip perpendicular to muscle fibre direction. Fatty deposits and connective tissue were avoided and SF was reported as the technical replicate average.

4.1.4 Particle size

Technical duplicate ~ 1.0 g samples were homogenised in 30 mL phosphate buffer (0.1 M KCl, 1 mM EDTA (di-sodium), 25 mM potassium phosphate) using an Ystral homogeniser (Series X10/25, Ystral, GER) set to 11,000 rpm (Silva et al., 2018). Particle size (PS) was then determined using a laser diffraction PS analyser (Model LS13-320, Beckman Coulter Ltd. Miami, USA) with a water connection and samples added drop-wise, in accordance with instrument guidelines, and average PS expressed as µm.

4.1.5 Sarcomere length

Thin slices (< 1 mm) removed parallel to the muscle fibre orientation from each samples. These were then analysed using laser-diffraction (Bouton et al., 1973) and the average from five technical replicates reported as sarcomere length (SL, µm).

4.1.6 Ultimate pH

Approximately 1.0 g samples were homogenised in 6 mL of the buffer solution (De Brito et al., 2016; Dransfield, 1994) using an Ystral homogeniser (Series X10/25, Ystral, GER) set to 19,000 rpm. These samples were then placed in a water bath where their temperature was allowed to equilibrate to 20 °C. A pH meter (smartCHEM-CP, TPS Pty Ltd., Brisbane, AUS) with a calibrated (at 20 °C using pH 4.0 and pH 6.8 standards) polypropylene spear-type gel electrode (Ionode IJ 44) was then used and the average of duplicate measures reported as the ultimate pH (pHu).

4.1.7 Colour stability

Sample slices (thickness: 3-4 cm) were placed onto individual black Styrofoam trays (area: 13.5 cm^2) so that muscle fibres on the exposure surface had perpendicular orientation. These were overwrapped with PVC food film (thickness: 15.0 µm) and held at 3-4 °C under continuous lighting (NEC 58 W Tubes delivering ~ 1000 lx to the sample surfaces, verified using a handheld lux meter). Overwrapped samples were periodically measured, a total of four times at daily intervals (0-3 d) – the first measures were made after an initial 30-40 min blooming period. A calibrated spectrophotometer (Model 45/0-L, Series No. 7237, HunterLab Associates Laboratory Inc., Hong Kong, PRC) with a 25 mm aperture and Illuminant D65 and 10° standard observer settings was used to measure sample CIE colorimetrics (L*, a* and b*) and reflectance spectra (Holman, Ponnampalam, van de Ven, Kerr, & Hopkins, 2015). These measurements were made in duplicate, with care to avoid connective tissue and fat deposits. Results were then used to calculate hue, chroma and the ratio of reflectance at 630 nm and 580 nm (R630/580) (AMSA, 2012).

4.1.8 Thiobarbituric acid reactive species

An adapted Hopkins et al. (2014) method was used, wherein ~ 100 mg samples were homogenised, using micro-tube pestles, with 500.0 µL radio immunoprecipitation assay (RIPA) buffer (no. 10010263, RIPA buffer concentrate, Cayman Chemicals Ltd., Michigan, USA). These were centrifuged and the supernatant absorbance measured at 532 nm using a benchtop spectrophotometer and as per the OXI-tek TBARS Assay Kit Technical Bulletin (no. ALX-850-287-Ki01, Enzo® Life Sciences Inc., New York, USA). Results were expressed as mg malondialdehyde (MDA) per kg fresh beef (mgMDA/kg).

4.1.9 Statistical analysis

Data were analysed using linear mixed models (Genstat 19th Edition, VSN International Ltd., www.vsni.co.uk) with cut, ageing period, grade and all relevant interactions fitted as fixed effects; and carcass (and cook batch for SF and CL) as random effects. The colorimetric traits were likewise analysed, except display and all its relevant interactions were also fitted as fixed effects so as to permit up to four-way interactions to be tested. pHu was included as a covariate for DL, SF, CL and all colorimetric traits. Differences between least square (predicted) means were judged to be significant if they exceeded the least significant difference at the 5% level.

4.2 Experiment Two

4.2.1 Sample collection

A total of 42 beef carcasses were selected at approx. 30 min *post-mortem*. These were immediately evaluated, with the striploin (HAM: 2140 (Anonymous, 2005); *M. longissimus lumborum*), bolar blade (HAM: 2302 (Anonymous, 2005); *M. triceps brachii*) and topside (HAM: 2001 (Anonymous, 2005); *M. semimembranosus*) pH and temperature values recorded using a pH meter (Model smartCHEMC-CP, TBS Ltd., Queensland, AUS), calibrated using pH 4.0 and pH 6.8 standards (at 2-3 °C). At this same juncture, sample cores (diameter: approx. 1 cm) were removed from the same three cuts; placed in correspondingly labelled tubes; and snap-frozen in liquid nitrogen, to halt further metabolism (Fig. 4.1). Following this initial collection, each carcass underwent an additional four collection intervals (total: 5) wherein this previous sampling procedure was repeated. The collection interval times were recorded as the total *post-mortem* duration (min), but were distributed somewhat evenly over the first 24 h – during which experimental carcasses were held in the chiller of a commercial Australian abattoir (temperature: approx. 4 °C).

Fig. 4.1
An example of sample core collection from the experimental beef carcasses

4.2.2 Glycogen determination

Approximately 20.0 mg samples were combined with 200.0 µL Milli-Q H_2O and incubated in a dry-heat block (set to 120 °C) for ~ 5 min, so that temperatures exceeded boiling point and all enzymatic processes were deactivated. These were then homogenised using micro-tube pestles; centrifuged; and the supernatant held on ice until analysis. Sample glycogen content was quantified as the average of technical duplicates and in accordance to the Glycogen Assay Kit Technical Bulletin (no. MAK016, Sigma-Aldrich Ltd., Missouri, USA) colorimetric protocol, using a benchtop spectrometer (model FLUOstar OPTIMA™, BMG Labtechnologies, Victoria, AUS) set to measure absorbance at 570 nm. Data was reported as mmol glycogen per kg of (wet) sample (mmol/kg).

4.2.3 Lactate determination

Samples (~ 20 mg) were homogenised with 200 µL Milli-Q H_2O, centrifuged and the supernatant was then analysed for its lactate content as per the Lactate Assay Kit Technical Bulletin (no. MAK064, Sigma-Aldrich Ltd., Missouri, USA) colorimetric protocol and using the same benchtop spectrometer, again set to measure absorbance at 570 nm. Data was expressed as mmol lactate per kg of (wet) sample, and the average of technical duplicates was recorded.

4.2.4 Glucose determination

Samples of ~ 20 mg were first homogenised in 200 µL MilliQ H_2O using micro-tube pestles, and then centrifuged so that the supernatant could be analysed and glucose content determined. This was undertaken as per the Glucose Assay Kit Technical Bulletin (no. MAK263, Sigma-Aldrich Ltd., Missouri, USA) colorimetric protocol, using a benchtop spectrometer set to measure absorbance at 570 nm. Data was reported as mmol glucose per kg of (wet) sample and the average of technical duplicates reported.

4.2.5 Glucose-6-Phosphate determination

G6P was determined in accordance to the Glucose-6-Phosphate Assay Kit Technical Bulletin (no. MAK014, Sigma-Aldrich Ltd., Missouri, USA) colorimetric protocol and the same benchtop spectrophotometer set to measure absorbance at 450 nm. Samples were first prepared – with the supernatant of centrifuged ~ 20 mg samples first homogenised in 200 µL MilliQ H_2O tested. Data was expressed as mmol glucose-6-phopsphate per kg of (wet) sample, and the average of technical duplicates was recorded.

4.2.6 Statistical analysis

Data were analysed using an exponential decay model as per Bruce, Scott, & Thompson (2001) under the software package *R* (R Core Team, 2018).

4.3 Experiment Three

4.3.1 On-carcass measures and sample collection

From the chiller of a commercial Australian abattoir, a total of 436 beef carcasses graded as DC (*n* = 42) or nDC (*n* = 394) were evaluated – reflective of the frequencies observed in the abattoir. Grading was undertaken in accordance to industry norm and at ~ 12-16 h *post-mortem* (MLA, 2017) – wherein the loin (HAM: 2140 (Anonymous, 2005); *M. longissimus lumborum*) surface between the 12-13th rib was exposed, allowed to bloom (~ 1.5 h at 2-3 °C), and evaluated so that carcasses with pH > 5.7 were considered DC. These same surfaces were then measured using a NIX (aperture: 15 mm; Nix Sensor Ltd., Ontario, CAN) with Illuminant D65 and 10° standard observer settings. Measures were taken at seven sites on the surface (Holman, Collins, Kilgannon, & Hopkins, 2018), with the colorimeter repositioned between each, taking care to avoid connective tissue and fat deposits. Colorimetric data were reported as the average hue, chroma and CIE values (L*, a* and b*) (CIE, 1978).

For a selection of experimental carcasses that represented DC and nDC (Table 4.1), the loin, topside (HAM: 2001 (Anonymous, 2005); *M. semimembranosus*) and bolar blade (HAM: 2302 (Anonymous, 2005); *M. triceps brachii*) were removed, individually vacuum-packaged, and aged for 14 d at 1-2 °C – on-site and with care to adhere to conventional industry practice. Samples were then transported to the Centre for Red Meat and Sheep Development (Cowra, New South Wales, AUS) where they were portioned for quality trait determination. These subsamples were held at – 25 °C prior to analysis, except for those measured for colour stability which were tested fresh.

4.3.2 Quality trait determination

Frozen sample blocks (mean ± standard deviation: 68.0 ± 5.3 g) were prepared and tested for their shear force (SF) and cooking losses (CL) in accordance with Holman, Van de Ven, Coombs, & Hopkins (2017). This used a texture analyser (Model LRX, Lloyd Instruments Ltd., Hampshire, UK) with attached Warner-Bratzler vee-shaped blade set to 200 mm crosshead speed. The average peak force (Newtons, N) applied to sever each of six technical replicate strips perpendicular to the muscle fibre orientation was recorded.

The Bouton et al. (1973) method for sarcomere length determination was applied, wherein five thin slices (thickness: < 1 mm) removed parallel to the muscle fibre orientation, evaluated using laser-diffraction, and their average results expressed as µm.

Ultimate pH were quantified using a pH meter (Model smartCHEMC-CP, TPS Ltd., Queensland, AUS) calibrated at pH 4.0 and pH 6.8 (at 20 °C). Samples were prepared and analysed as per De Brito et al. (2016) with the results expressed as the average of technical duplicates.

Drip loss (DL) results were calculated as the percentage weight change of a sample core (dimensions: 25 mm^2) held under refrigeration (3-4 °C) for 3 d and within an EZ-Drip container (Danish Meat Research Institute, Taastrup, DEN).

Colour stability was measured on sample slices (thickness: 3-4 cm) which were placed onto black Styrofoam trays (dimensions: 13.5 cm^2) so that the muscle fibres on the exposed surface were orientated perpendicular underneath the PVC over-wrap (food film thickness: 15.0 µm). These were permitted to bloom for 30-45 min at 1-2 °C before the first colour measurement was recorded (0 d).

A further 3 measurements (1, 2 and 3 d) were taken at 1 d intervals (four in total) between which samples were held at 3-4 °C and under continuous florescent lighting (58 W NEC Tubes delivering approximately 1000 lux, monitored using a handheld lux and foot-candles meter). All colour measurements (CIE values: L*, a* and b*; Chroma and hue) were recorded as the average of seven technical replicates and using the same NIX (aperture: 15 mm; Nix Sensor Ltd., Ontario, CAN) set to Illuminant D65 and 10° standard observer (Holman et al., 2018).

4.3.3 Statistical analysis

The carcass grade of each sample was coded as DC = 1 and nDC = 0 to enable a quantitative analysis of DC probability predicted by the various colour measurements. Logistic regression models were fitted to estimate DC probability in response to each colour parameter. A decision rule for classification can be developed by an arbitrary probability threshold so that carcases with estimated probabilities above the threshold are rated DC. Study of specificity (proportion of correctly classified DC carcases) and sensitivity (proportion of correctly classified nDC carcases) as the threshold changes via Receiver Operator Characteristic (ROC) curve fitting yielded an optimum classification rule at the point where the sum of sensitivity and specificity was maximised. The area under the ROC curve for each colour parameter was used to identify the best predictors of DC class as per the methods described in Robin, Turck, & Hainard (2011).

The degree of association between each meat quality trait and each colour measurement within each cut was measured by fitting simple linear regression models. Statistical importance of each predictor was assessed by the F-ratio test for variance accounted for by each model. All graphics and analyses were constructed in the *R* environment (R Core Team, 2018).

5.0 PROJECT OUTCOMES

5.1 Experiment One

Fixed effects and their interactions were not found to be significant unless explicitly included hereafter:

5.1.1 Drip loss

There was a lower DL ($P < 0.001$) for cuts aged 28 d (0.85 ± 0.09 %) than those aged for 14 d (1.43 ± 0.09 %). Of the cuts, the topside had higher DL ($P < 0.05$) than the striploin, and neither of these cuts had DL values that were significantly different to that observed for the bolar blade (Fig. 5.1).

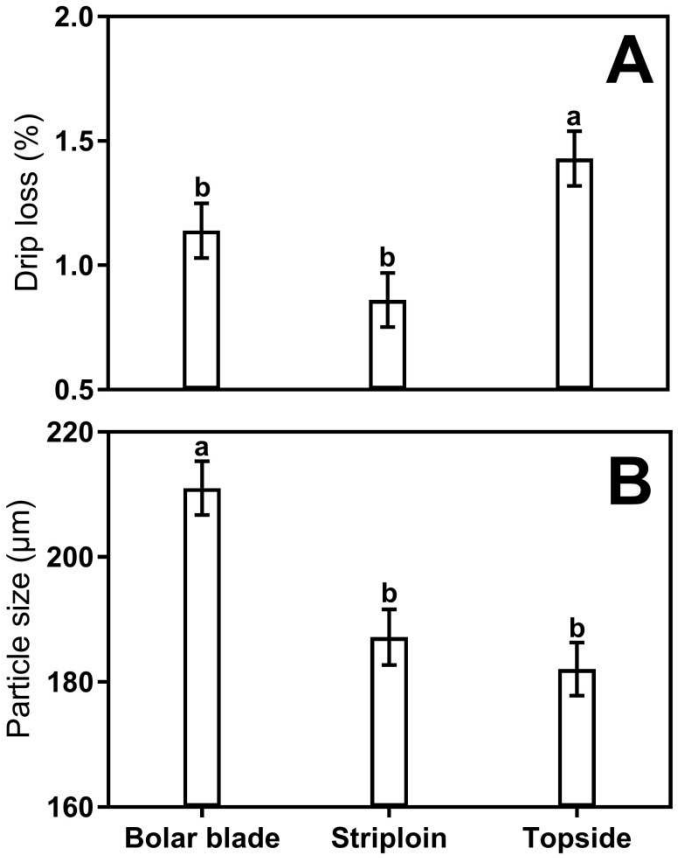

Fig. 5.1.

The predicted mean (± standard error) A) drip loss (%); and B) particle size (µm) of beef bolar blade, striploin and topside cuts. Columns with different letters are significantly different ($P < 0.05$).

5.1.2 Cooking loss and shear force

Cut and ageing period interactions on CL were significant ($P < 0.001$; Table 5.1) as the bolar blade was observed to lose more fluid during cooking than the topside and striploin, when aged for 28 d. Cuts aged for 14 d instead demonstrated no difference in CL between the bolar blade and topside. There was also no difference in the extent of fluid loss with ageing the bolar blade, whereas by contrast much less fluid was lost by the striploin and topside with ageing. There was an effect of pHu ($P < 0.001$) such that cooking loss decreased by 6.3 ± 0.8 % for every pH unit increase. No effect of grade on CL was found ($P > 0.05$).

Table 5.1

Predicted means (± standard error) for cooking losses (%) according to cut and ageing period (14 d and 28 d).[1]

Traits	Ageing Period (d)	Cut		
		Bolar blade	Striploin	Topside
Cooking loss (%)	14	21.7 ± 0.57^{ax}	18.7 ± 0.58^{ay}	22.0 ± 0.56^{ax}
	28	21.2 ± 0.57^{ax}	13.0 ± 0.58^{by}	17.6 ± 0.58^{bz}

[1]Trait means within columns with differing superscripts (a, b) were significantly different ($P < 0.05$). Trait means within rows with differing superscripts (x, y, z) were significantly different ($P < 0.05$).

There was an interaction ($P < 0.05$) between cut and grade for SF such that the DC topsides were tougher than the striploins and bolar blades, which were not different. However for the nDC samples the striploin was less tough ($P < 0.05$) than the other two cuts which were not different (Table 5.2). There was an effect of pHu ($P < 0.05$) such that SF decreased by 6.2 ± 2.5 N for every pH unit increase. There was no effect of ageing ($P > 0.05$) with means for 14 and 28 d aged product of 34.3 ± 0.9 N and 32.7 ± 0.9 N respectively.

Table 5.2

Predicted means Predicted means (± standard error) for ultimate pH (pHu), sarcomere length, shear force, b*, R630/580 and hue according to cut and carcass grade (dark cutting [DC] and control [nDC]).[1]

Traits	Grade	Cut		
		Bolar blade	Striploin	Topside
pHu	DC	5.61 ± 0.04^{ax}	6.21 ± 0.04^{ay}	5.73 ± 0.04^{ax}
	nDC	5.61± 0.04^{ax}	5.57± 0.04^{bxy}	5.47± 0.04^{by}
Sarcomere length (µm)	DC	2.52 ± 0.08^{ax}	1.77 ± 0.08^{ay}	1.70 ± 0.08^{ay}
	nDC	2.25 ± 0.08^{bx}	1.96 ± 0.08^{ay}	1.87 ± 0.08^{ay}
Shear force (N)	DC	33.9 ± 1.32^{ax}	30.4 ± 1.83^{ax}	42.4 ± 1.31^{ay}
	nDC	34.1 ± 1.41^{ax}	25.4 ± 1.43^{by}	35.0 ± 1.52^{ax}
b*	DC	17.1 ± 0.27^{ax}	14.2 ± 0.35^{ay}	15.8 ± 0.26^{ay}
	nDC	16.9 ± 0.28^{ax}	14.1 ± 0.29^{by}	17.1 ± 0.30^{ax}
R630/580	DC	5.3 ± 0.11^{ax}	4.8 ± 0.15^{ay}	5.2 ± 0.11^{ax}
	nDC	5.2 ± 0.12^{ax}	4.3 ± 0.12^{by}	4.7 ± 013^{bz}
hue	DC	38.4 ± 0.59^{ax}	39.1 ± 0.80^{ax}	37.7 ± 0.57^{ax}
	nDC	38.1 ± 0.62^{bx}	38.5 ± 0.63^{bx}	41.3 ± 0.66^{by}

[1]Trait means within columns with differing superscripts (a, b) were significantly different ($P < 0.05$). Trait means within rows with differing superscripts (x, y, z) were significantly different ($P < 0.05$).

5.1.3 Particle size

When pHu was fitted as a covariate ($P < 0.001$), the topside PS value was the highest (211.0 ± 4.30 µm; $P < 0.05$) and sequentially followed by the striploin and bolar blade cuts which did not themselves differ (Fig. 5.1). Ageing period was significant with PS observed to be higher at 14 d (197.8 ± 3.70 µm) compared to 28 d (189.1 ± 3.73 µm). All other main or second order interaction effects on PS were found to be insignificant ($P > 0.05$).

5.1.4 Sarcomere length

For SL there was an interaction ($P < 0.05$) between cut and grade such that the bolar blade was shown to have a much longer SL than found for the remaining cuts (Table 5.2) – although this difference was lesser when bolar blades were graded as nDC compared to DC.

5.1.5 Ultimate pH

There was an interaction ($P < 0.001$) between cut and grade for pHu such that for both the striploin and topside the values were higher for DC specified cuts (Table 5.2). Additionally, the striploin had the highest pHu of all DC cuts, but the other two cuts were not significantly different, whereas for the nDC cuts, the topside had a lower pHu than the bolar blade, but not the striploin (Table 5.2).

5.1.6 Colour stability

No four-way interaction was observed between grade, cut, ageing period and display period ($P > 0.05$) for any colorimetric trait.

For L* there was an independent effect ($P < 0.001$) of cut, grade, ageing period and display period (Fig. 5.2), with all interactions were observed to be insignificant. DC samples were darker (lower L* values) than their nDC counterparts. Samples aged for 28 d were lighter ($P < 0.001$) than those aged for 14 d, although the numerical difference was not large. The topside had lower L* and was therefore darker (37.3 ± 0.31; $P < 0.001$) than both the bolar blade and striploin (38.5 ± 0.31 and 38.0 ± 0.31, respectively). There was an inconsistent trend in the L* values during display – with L* values at 1 d display period observed to be the highest. There was a covariate effect of pHu ($P < 0.001$) wherein L* values decreased by 6.4 ± 0.48 units for every pH unit increase.

There was a three-way interaction ($P < 0.001$) between cut, ageing period and display period for sample redness (a*) with the highest values observed for the bolar blade which then reduced across display period – albeit to a lesser degree for beef aged for 14 d compared to that aged for 28 d (Table 5.3). There were lower a* values for longer aged product (28 d) and after 1 d of display, the striploin had dropped to below the consumer acceptance threshold level of a* ≥ 14.5 (Holman, van de Ven, Mao, Coombs, & Hopkins, 2017), with the bolar blade showing the most stable values under display. There was an effect of pHu ($P < 0.001$) such that a* values decreased by 6.6 ± 0.45 units for every unit increase in pH.

Likewise, there was a three-way interaction ($P < 0.001$) between cut, ageing period and display period for yellowness as the bolar blade exhibited the highest b* values (Table 5.3) and then, more so in samples aged for 28 d. The striploin had the lowest b* values at the 28 d ageing period, with the topside intermediate to the bolar blade and striploin. When aged for 14 d, there was no difference between the bolar blade and topside over the display period. There was a two-way interaction ($P < 0.001$) between grade and cut, such that the DC bolar blade had higher b* values (Table 5.2) than both the striploin and topside, which were not different; and, for nDC samples there was no difference between the bolar blade and topside for b* values. There was a covariate effect of pHu ($P < 0.001$) such that b* values decreased by 5.3 ± 0.47 units for every unit increase in pH.

R630/580 was found to exhibit similar (third-order interaction) effects as b*. The bolar blade and topside presented a R630/580 decline as display period increased and irrespective of ageing period (Table 5.3). The striploin, when aged for 14 d, had minimal R630/580 changes across display, contrasted with an observed display period decline ($P < 0.001$) for 28 d aged striploin. The striploin R630/580 values were lowest when aged for 28 d. There was also a two-way interaction ($P < 0.001$) between grade and cut, such that DC and nDC striploins had lower R630/580 values (Table 5.2) and the nDC topside had lower values than the equivalent bolar blade. There was an effect of pHu ($P < 0.001$) such that ratio values decreased by 1.11 ± 0.22 units for every unit increase in pH.

Table 5.3

Predicted means and standard errors (Std.error) for a*, b*, the reflectance ratio of 630 nm and 580 nm (R630/580), chroma and hue according to cut, ageing period (14 d and 28 d) and display period (0-3 d).[1]

Trait	Cut	Ageing period (d)	Display period (d) 0	1	2	3	*Std.error*
a*	Bolar blade	14	23.6^{awy}	23.7^{aw}	22.0^{bcw}	21.2^{cw}	0.51
	Striploin		20.1^{ax}	23.1^{bwx}	22.9^{bw}	22.7^{bx}	
	Topside		22.6^{aby}	23.5^{aw}	21.9^{bcw}	20.7^{cw}	
	Bolar blade	28	23.2^{awy}	21.8^{bx}	20.5^{bx}	18.4^{cy}	0.51
	Striploin		21.6^{az}	13.8^{by}	11.8^{cy}	11.0^{cz}	
	Topside		22.1^{azy}	18.8^{bz}	17.1^{cz}	15.0^{dv}	
b*	Bolar blade	14	18.4^{aw}	17.4^{bw}	17.0^{bwx}	16.9^{bw}	0.39
	Striploin		15.9^{ax}	16.7^{aw}	16.9^{awx}	16.6^{aw}	
	Topside		17.9^{awy}	17.6^{abw}	17.5^{abw}	16.9^{abw}	
	Bolar blade	28	17.3^{ay}	16.8^{aw}	16.6^{abx}	15.6^{bx}	0.39
	Striploin		14.1^{az}	11.4^{bz}	10.8^{bz}	11.1^{bz}	
	Topside		17.3^{ay}	15.1^{by}	15.2^{by}	14.2^{by}	
R630/580	Bolar blade	14	7.0^{aw}	5.7^{bw}	5.1^{cdw}	4.7^{dw}	0.18
	Striploin		5.8^{ax}	5.7^{aw}	5.7^{axy}	5.3^{bx}	
	Topside		6.5^{ayv}	5.7^{bw}	5.3^{bwy}	4.6^{cw}	
	Bolar blade	28	6.2^{axyv}	5.1^{bx}	4.6^{cz}	4.0^{dy}	0.18
	Striploin		4.9^{az}	3.3^{by}	2.8^{cv}	2.7^{cz}	
	Topside		6.3^{av}	4.4^{bz}	3.8^{cu}	3.3^{dv}	
Chroma	Bolar blade	14	30.0^{aw}	29.4^{aw}	27.8^{bwx}	27.1^{bw}	0.59
	Striploin		25.6^{ax}	28.5^{bwx}	28.4^{bw}	28.1^{bw}	
	Topside		28.9^{awy}	29.3^{aw}	28.0^{abw}	26.8^{bw}	
	Bolar blade	28	29.0^{awy}	27.6^{abx}	26.4^{bx}	24.2^{cx}	0.59
	Striploin		25.8^{ax}	18.1^{by}	16.2^{cy}	15.8^{cy}	
	Topside		28.1^{ay}	24.2^{bz}	22.9^{bz}	20.8^{bcz}	
Hue	Bolar blade	14	38.2^{aw}	36.5^{aw}	37.8^{aw}	38.9^{awy}	1.00
	Striploin		37.5^{aw}	35.2^{aw}	35.8^{aw}	35.6^{ax}	
	Topside		38.2^{aw}	36.6^{aw}	38.5^{awx}	39.1^{awy}	
	Bolar blade	28	37.0^{aw}	37.8^{abwx}	39.3^{abx}	40.4^{by}	1.00
	Striploin		32.3^{ax}	40.1^{bx}	48.5^{cy}	45.5^{dzv}	
	Topside		38.3^{aw}	39.3^{ax}	42.0^{bz}	43.9^{bv}	

[1]Trait means within rows with differing superscripts (a, b, c, d) were significantly different ($P < 0.05$). Trait means within columns with differing superscripts (u, v, w, x, y, z) were significantly different ($P < 0.05$).

There was a three-way interaction ($P < 0.001$) between cut, ageing period and display period for chroma (Table 5.3). Chroma values for the bolar blade declined with increasing display period and irrespective of ageing period, whereas for the striploin there was minimal change for 14 d aged samples, but a large chroma decline observed for the 28 day aged counterparts. The topside followed a similar trend to the striploin, whereby the decline in values was not as obvious for 28 d aged product during display. There was an effect of pHu ($P < 0.001$) such that Chroma values decreased by 8.3 ± 0.52 units for every pH unit increase.

Again, there was a three-way interaction ($P < 0.001$) between cut, ageing period and display period for hue (Table 5.3). The striploin aged for 28 d showed an increase in hue with display period and then decreased at 3 d display period. The topside exhibited an increase in hue with display when aged for 28 d (Table 5.3). A higher hue value was found for all cuts after 3 d display periods and when these were aged for 28 d rather than 14 d. There was also a two-way interaction ($P < 0.001$) between grade and cut, such that DC topsides had higher hue values than nDC topsides (Table 5.2) – nDC topside having higher hue values than both the striploin and bolar blade. Samples aged for 14 d and were DC had lower hue values (36.4 ± 0.54) than those aged for 28 d (40.4 ± 0.52). nDC samples had highest hue values irrespective of ageing period (38.2 ± 0.52 and 40.3 ± 0.59, respectively) for 14 d and 28 d. There was an effect of pHu ($P < 0.001$) such that hue values increased by 2.51 ± 1.16 units for every unit increase in pH.

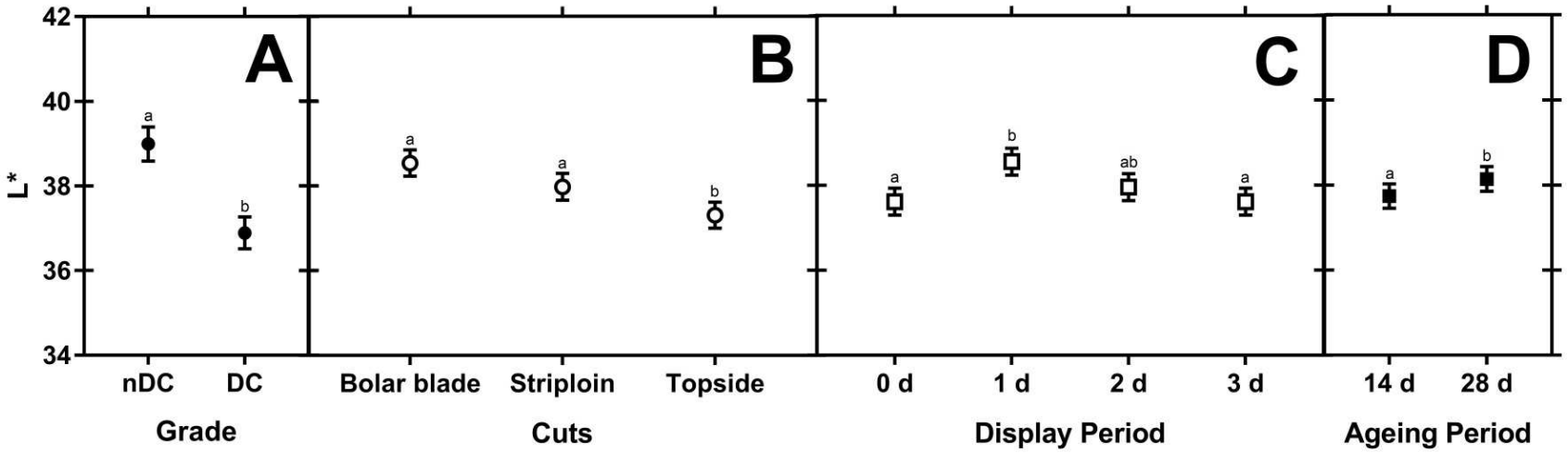

Fig. 5.2.

The predicted mean (± standard error) of the independent effects of A) grade; B) cuts; C) display period; and D) ageing period on sample L* values. Symbols with different letters within each effect are significantly different ($P < 0.05$).

5.1.7 Thiobarbituric acid reactive species

The striploin TBARS content was lower (0.75 ± 0.06 mgMDA/kg; $P < 0.001$) than the bolar blade (1.07 ± 0.06 mgMDA/kg) and topside (1.04 ± 0.06 mgMDA/kg), which themselves did not significantly differ. Only DC sample TBARS content was found to increase with ageing period ($P < 0.05$). These same DC samples also had lower TBARS content than nDC and this difference was observed for each ageing period ($P < 0.05$; Table 5.4).

Table 5.4

Predicted means (± standard errors) for thiobarbituric acid reactive species (TBARS) according to ageing period (14 and 28 d) and grade (dark cutting [DC] and control [nDC]).[1]

Trait	Ageing period (d)	Grade	
		DC	nDC
TBARS (mgMDA/kg)	14	0.72 ± 0.10^{ax}	1.12 ± 0.10^{b}
	28	0.90 ± 0.10^{by}	1.07 ± 0.10^{a}

[1]Trait means within rows with differing superscripts (a, b) were significantly different ($P < 0.05$). Trait means within columns with differing superscripts (x, y) were significantly different ($P < 0.05$).

5.2 Experiment Two

5.2.1 pH decline

The pH values for all cuts were shown to decrease over time. The striploin and topside pH declined respectively at 0.4% and 0.5% per hour which were different to the bolar blade decline of 0.6% per hour – it noteworthy that the bolar blade pH levels were initially highest (Fig. 5.2).

5.2.2 Glycolysis traits

Across the first 24 h *post-mortem*, bolar blade lactate content increased 1.3% per hour ($P < 0.001$). Likewise in the bolar blade, glycogen ($P < 0.001$) and glycolytic potential ($P < 0.001$) increased 1.6% and 1.2% per hour respectively over this same time period. The bolar blade glucose content instead was observed to decrease 1.2% per hour ($P < 0.05$). G-6-P also tended to decrease over time, but this was not significant when carcass and muscle temperature variation was accounted (Fig. 5.3). For the striploin, all glycolysis traits tended to increase in the striploin with the exception of glucose and glycogen which instead decreased across the total time period evaluated (Fig. 5.3). However, when we accounted for the carcass and muscle temperature within carcass variation, we observe variation over time as significant only for lactate which on averaged increased 1.2% per hour ($P < 0.01$), and for glucose which instead declined 0.58% per hour on average ($P < 0.05$). All glycolysis traits declined in the topside over the first 24 h *post-mortem* with the exception of lactate which was not influenced by time ($P > 0.05$). Glucose declined 1.9% per hour ($P < 0.001$), and glycogen ($P < 0.05$), G-6-P ($P < 0.01$) and glycolytic potential ($P < 0.05$) each declined at 0.1% per hour ($P < 0.01$). It should be noted that modelling permitted these trends to be extrapolated up to 30 h *post-mortem* to demonstrate the continuation in the rate of glycolysis metabolite variation over time (Fig. 5.3).

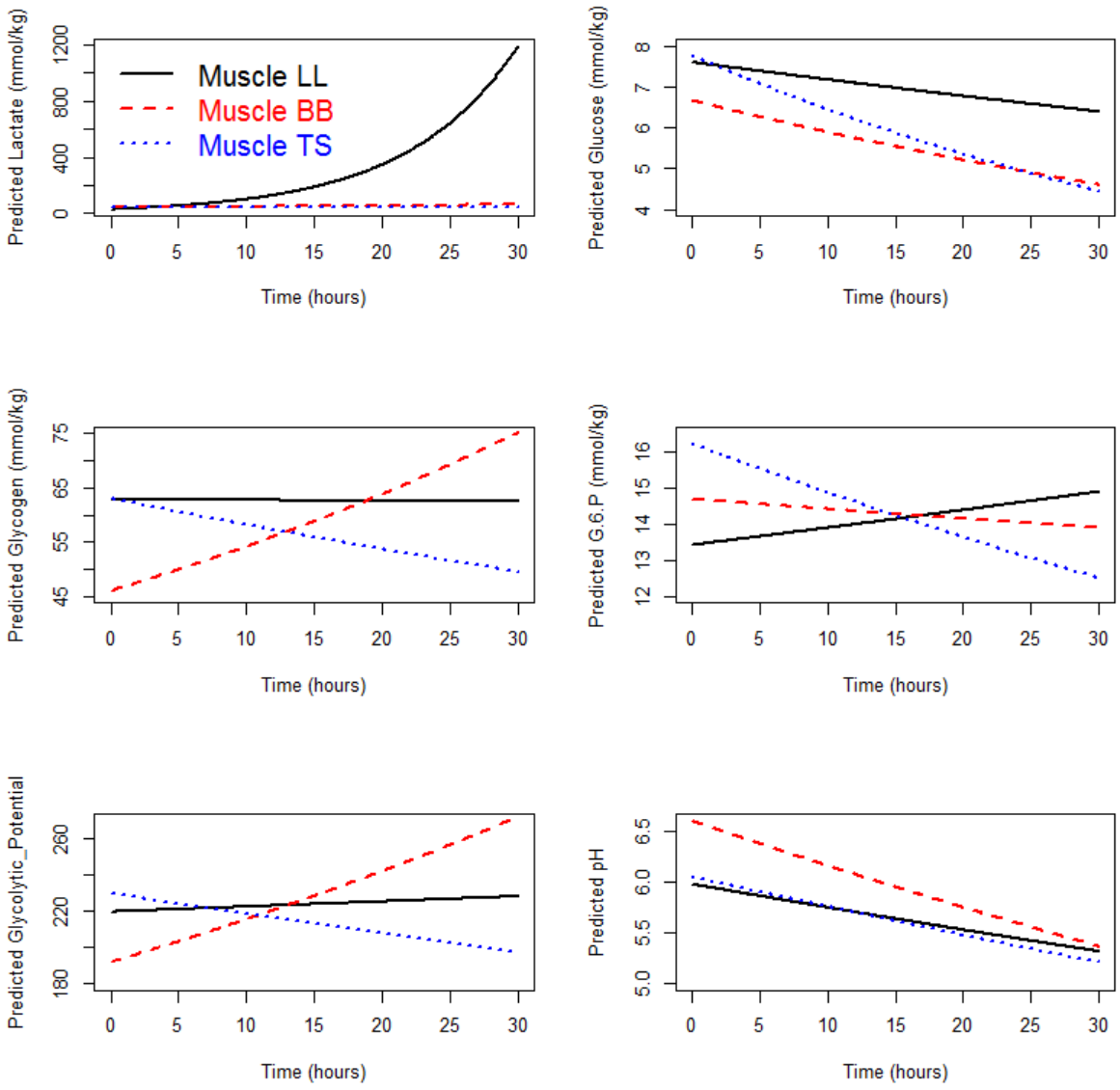

Fig. 5.3

Predicted pH and glycolysis metabolite variation across the first 30 h *post-mortem* measured in beef striploin (LL), bolar blade (BB) and topside (TS) cuts. Please note that glucose-6-phosphate is abbreviated as G-6-P in the corresponding graph.

5.3 Experiment Three

The results from this experiment merited incorporation within their corresponding discussion section and can be found in **Section 6.3**.

Table 5.5

Summary data from the receiver operator characteristic (ROC) analysis of classification into carcass grade (dark cutting and control/non-dark cutting) by each NIX recorded colorimetric.[1]

Colorimetric	AUC	AUC 95% Confidence Intervals	Threshold	Threshold value	Sensitivity	Specificity
Chroma	0.94	(0.90,0.98)	0.12	30.54	0.91	0.86
b*	0.94	(0.90,0.98)	0.23	11.91	0.94	0.81
L*	0.92	(0.88,0.96)	0.10	38.64	0.86	0.81
a*	0.88	(0.81, 0.94)	0.21	18.76	0.92	0.71
hue	0.71	(0.63,0.79)	0.08	32.57	0.54	0.81

[1]Colorimetric order was sorted from highest to lowest area under ROC curve (AUC).

Table 5.6

Residual standard error (RSE), coefficients of determination (R^2) and F-ratio test for variance explained (*P* value) from linear models to predict beef quality traits of the bolar blade, striploin and topside (cuts) with NIX chroma values recorded in-plant, at grading.

Quality trait	Bolar blade			Striploin			Topside		
	RSE	R^2	*P value*	RSE	R^2	*P value*	RSE	R^2	*P value*
Sarcomere length (µm)	0.46	0.09	0.109	0.19	0.07	0.153	0.22	0.01	0.711
pHu	0.07	0.06	0.190	0.31	0.23	0.007	0.23	0.23	0.001
Drip loss (%)	0.66	0.07	0.169	0.71	0.11	0.076	1.58	0.00	0.929
Cooking loss (%)	2.33	0.00	0.812	3.13	0.12	0.057	3.24	0.08	0.123
Shear force (N)	4.96	0.02	0.444	5.67	0.07	0.171	8.86	0.07	0.148
ΔE[1]	3.20	0.04	0.303	6.03	0.00	0.963	2.70	0.00	0.871

[1]ΔE: The colour change between 0 d and 3 d of display (AMSA, 2012).

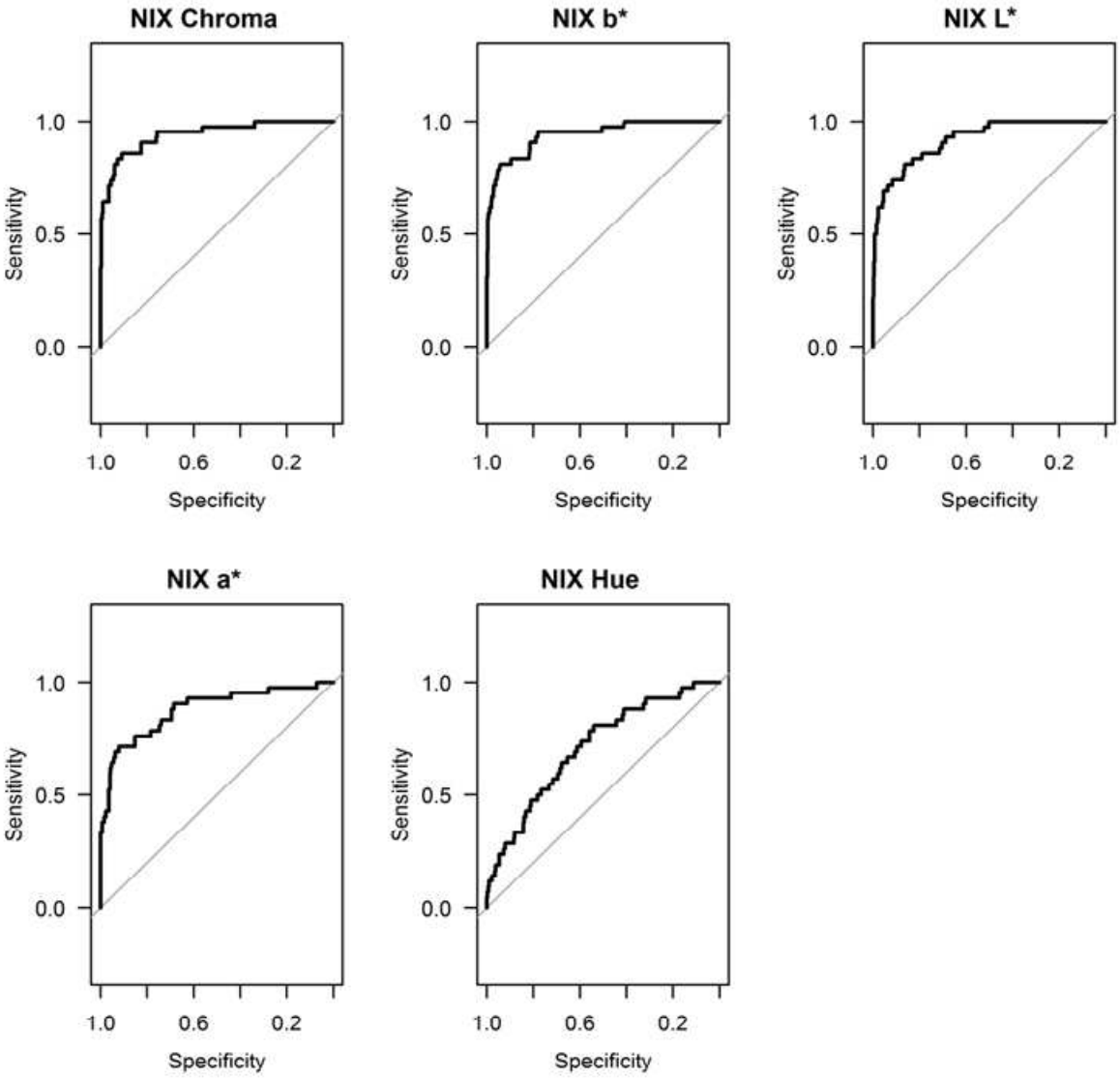

Fig. 5.4

Receiver operator characteristic (ROC) curves for carcass grading using L*, a*, b*, chroma and hue colorimetrics.

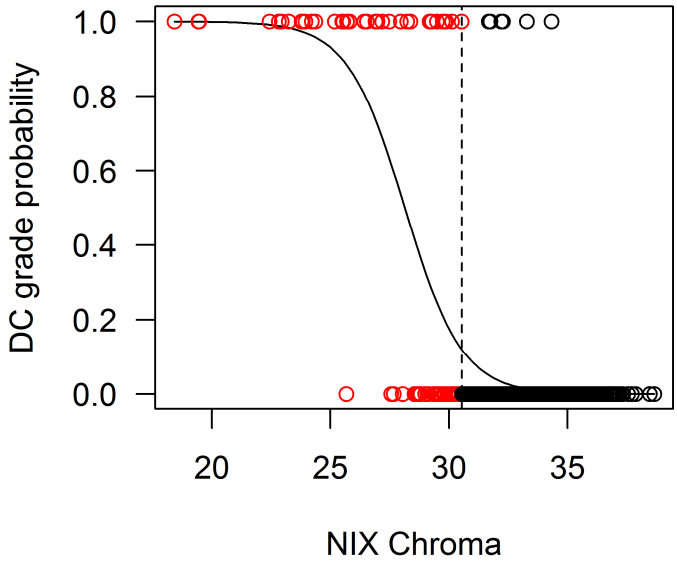

Fig. 5.5

The observed grade (DC=1, nDC=0) and estimated probability of carcass grade being dark cutting (DC grade) as related to chroma. The receiver operator characteristic (ROC) estimated optimum threshold is indicated by the vertical dashed line. Colours show the resulting classification into DC (red) and control/non-dark cutting (black).

6.0 DISCUSSION

6.1 Experiment One

The effects of carcass grade on the tenderness traits were not uniform for all three evaluated cuts, as the bolar blade failed to match the increased toughness results observed in the topside and striploin from DC versus nDC carcasses. This suggests that the bolar blade, and potentially the forequarter (Bass et al., 2008; Prieto, Lopez-Campos, Suman, Rodas-Gonzalez, & Aalhus, 2018), of DC beef carcasses could be recovered and attract the same market value as their nDC equivalents.

This finding was supported by the pHu results that mirror the tenderness disparity between cuts, because tenderness is a function of meat pH. This relationship is based on the impact of pH on enzymatic and metabolic functionality, and consequently myofibril structure and its resistance to fragmentation/chewing. Furthermore, past research has reported similar variation between cuts and muscles within beef carcasses. Wulf, Emnett, Leheska, & Moeller (2002) found SF results differed between nDC and DC *m. longissimus lumborum* and *m. semimembranosus*, but not for the *m. psoas major*, *m. biceps femoris* or *m. rectus femoris*. Belew, Brooks, McKenna, & Savell (2003) identified significant variation both between and within several different bovine muscles based on their shear force values. Shackelford, Wheeler, & Koohmaraie (1995) also reported differences between ten different beef muscles, but found tenderness variation to be greatest in the *m. longissimus lumborum* with its results having differing strengths of correlation to the other muscles. Furthermore, in the Bass et al. (2008) study of muscle pH distribution in beef classified as DC (as per the USDA grading system whereby LL pH must be > 6) they found comparable results wherein forequarter muscles did not reflect the pH characteristics of the *m. longissimus lumborum* and hindquarter muscles. That said, based on the SF results, the general quality and acceptability of all cuts evaluated was observed. This could result from the severity of DC carcasses captured in the present study, with past research classifying mean pH = 6.1 as only 'shady DC' and pH = 6.9 as 'severe DC' (Grayson et al., 2016). Alternatively, it could result from the ageing period applied herein and its effects on organoleptic quality traits.

Ageing beef is often used to optimise eating quality (Coombs, Holman, Friend, & Hopkins, 2017), however only the TBARS content was found to increase in aged DC beef aged for 28 d when compared to samples aged for 14 d. Again, it merits attention that all TBARS values were below the levels previously reported to impact consumer satisfaction (Campo et al., 2006; Hughes, McPhail, Kearney, Clarke, & Warner, 2015). No other ageing period interactions with carcass grade were observed. This was somewhat expected as previous research has reported DC beef to have increased susceptibility to oxidation due to its increased activation of respiratory biochemical reactions and availability of extracellular water (water-holding content) in which oxidation can occur (Mahmood, Turchinsky, Paradis, Dixon, & Bruce, 2018; Talmant & Monin, 1986). Paradoxically, it was interesting that these differences did not translate to further TBARS differences across the ageing periods tested.

A possible basis for the absence of additional carcass grade and ageing period interactions would be the > 14 d duration used and its effects on myofibril structures being sufficient to 'mask' the initial differences between DC and nDC beef. As found in the present study, past research has shown variation in beef particle size and myofibril fragmentation indexes (MFI) to occur from ageing (Silva et

al., 2018). This may have disrupted the effects DC has on muscle fibre width and water-holding capacities (Hughes, Clarke, Purchas, & Warner, 2017) while also compartmentalising or potentially exhausting enzymatic activities (Lomiwes, Farouk, Wu, & Young, 2014). Again, the extent of this effect is not standard across all muscles, with an observed difference between nDC and DC bolar blade SL and Bruas-Reignier & Brun-Bellut (1996) demonstrating proteolysis differences between the *m. longissimus lumborum* and *m. triceps brachii* expressed as MFI and meat nitrogen content differences. Furthermore, these effects may also have prompted the absence of ageing period and carcass grade interactions on beef colour stability.

Colorimetric (b*, hue and R630/580) variations due to carcass grade were observed more so for the striploin than the other cuts, and this disparity was not influenced by ageing period or 3 d of retail display. An effect of carcass grade on colour is not unexpected as much research confirmed colour differences between DC and nDC beef (Ponnampalam et al., 2017). Hughes et al. (2017) reported that beef muscles with high pH had lower lightness, redness and 'global brightness' than samples with lower pH; and these, with myoglobin oxidative potentials, infer the discolouration associated to DC. Consequently, the pH differences previously discussed in reference to tenderness findings may also underpin the colour results herein. This cut difference is further supported by Wulf et al. (2002), that similarly found colour variation between DC and nDC carcasses aged for 7 d, but also observed these colour differences were limited to the striploin and topside as the *m. psoas major*, *m. biceps femoris* and *m. rectus femoris* did not reflect these colour variations. Likewise, Holdstock, Aalhus, Uttaro, Larsen, & Bruce (2012) noted Japanese Meat Grading Association colour score differences between the muscles of DC and nDC beef – concluding that the forequarter cuts did not reflect the discolouration evident in the middle and hindquarter cuts. Together, these support the tenderness observations that the forequarter (including the bolar blade) does not necessary mimic striploin characteristics and can be considered independently.

It should be acknowledged that independent to carcass grade; cut, ageing period and their interactions did influence eating quality, water-holding capacity and colour stability traits. Based on past research, this outcome should be expected and the background for these effects has been extensively reviewed (Coombs et al., 2017; Kim et al., 2018; Pearce, Rosenvold, Andersen, & Hopkins, 2011; Polkinghorne & Thompson, 2010). This result is still important as it provides credence to the value of a cuts based grading system, advantages of ageing for improved retail-potential and consumer appeal, and observation of within carcass grade effects.

6.2 Experiment Two

We observed that glycolysis continued up to 24 h *post-mortem* without trending towards stability for each of the three cuts investigated, the exception being the bolar blade and topside lactate contents. This suggests that dynamic beef carcasses are subject to grading under conventional Australian practices wherein assessment occurs between 14-16 h *post-mortem* somewhat premature to its ultimate glycolytic status. This outcome does reflect past research. Bendall (1979) found lactate and pH variations continued even at 48 h for the beef *triceps brachii*, *longissimus dorsi* and *biceps femoris* muscles. As per this experiment, O'Halloran, Troy, & Buckley (1997) reported pH declines continued in the *longissimus dorsi* and *semimembranosus* muscles throughout the first 24 h *post-mortem*. Furthermore, Tarrant & Mothersill (1977) observed *post-mortem* differences in pH decline rates for six beef muscles (within-carcass) that persisted, with the last measurement recorded at 48 h *post-*

mortem. The practical implications for these findings are best illustrated in the 5% reduction in dark cutting beef incidence when the time of grading was delayed from 14 h to 31 h *post-mortem* (Hughes, Kearney, & Warner, 2014). While this outcome highlights the quality and value implications of early beef carcass grading, it is worth noting that only the striploin was assessed and the rate of glycolysis is not uniform across the muscles of a carcass.

The *post-mortem* variation in glycolysis metabolites and pH was not consistent for the three cuts evaluated in this study. This is likely a reflection of their metabolic profile, and their fibre type (Ferguson & Gerrard, 2014). For example, Ylä-Ajos, Ruusunen, & Puolanne (2006) found beef *longissimus dorsi* and *masseter* muscle differences in terms of their glycolytic potentials; and commented on this being the result of their respective predominance of fast twitch and glycolytic muscle fibres versus slow twitch and oxidative muscle fibres. There are even differences within more similar fibre types, with muscle high in IIa fibres which are fast-twitch glycolytic and have higher glycogen contents than muscles predominantly IIb fibre types that are also fast-twitch but glycolytic-only (Pethick et al., 1999). Essentially, therefore, fibre type variations between muscles can be observed as pH or glycolytic potential differences. As the Talmant & Monin (1986) analysis of beef muscles sampled at 30 min *post-mortem* demonstrated striploin and topside glycolytic potentials were themselves comparable, and both proved to have higher potentials than observed for the bolar blade. This same study also found comparable striploin and topside pH values which, this time were both lesser than that observed for the bolar blade (Talmant & Monin, 1986). These outcomes reflect those found in the present study. Furthermore, as glycolysis metabolites and pH are associated with beef organoleptic and colour stability traits (Ferguson & Gerrard, 2014; Fischer & Hamm, 1979; Honikel, 2014) – we can observe a corroboration of previous results wherein the bolar blade from DC carcasses was not found to reflect the meat quality traits of either the striploin or topside (Experiment 1).

We found that muscle temperature contributions to the precision of glycolysis metabolite and pH models to be substantive. However, this outcome is not unique. Nuss & Wolfe (1981) also reported *post-mortem* glycogen, G-6-P, and pH variations to be more so a function of muscle temperature than muscle type, and result from temperature effects on glycolytic enzyme activity. Bendall (1977) supports this, finding the pH decline differences between four beef muscles was due to different rates of cooling between muscles within the same carcass. That said, this variability was still apparent even after data was corrected to a constant temperature (Bendall, 1977). Tarrant & Mothersill (1977) noted the rate of glycolysis increased with the depth at which the sample was collected; unsurprisingly therefore, a good relationship between muscle temperature and glycogen metabolism was also identified. In practical situations, carcass characteristics (i.e. fat cover, hot carcass weight or muscle dimensions) and chiller type and settings influence muscle temperature and therefore their rate of glycolysis. This has already been shown to reduce the incidence of pale, soft and exudative (PSE) in pork if appropriately managed (Savell, Mueller, & Baird, 2005).

These outcomes from this experiment should be interpreted with recognition that glycolysis was monitored with each cut held *in situ* and within the same chiller. Under these conditions the bolar blade glycolytic metabolite trends were different to the striploin and topside. This could suggest, therefore, that the forequarter be removed and held under a more suitable chilling approach so as to efficiently achieve uniform rate of glycolysis and associated quality traits – in whole-carcass terms. That said it is important that the practical value of any such approach is confirmed prior to adoption,

notably because of the inherent differences between beef carcasses.

The rate of glycolysis was found to differ between individual carcasses and doing so support the notion that pre-slaughter factors can deplete or increase an animals glycogen stores. These factors have been the topic of much review and research (Ferguson & Warner, 2008; Lahucky, Palanska, Mojto, Zaujec, & Huba, 1998; Maher et al., 2004; McGeehin, Sheridan, & Butler, 2001; Ponnampalam et al., 2017). Indeed, Bendall (1977) reported that the lactate content differences for the six experimental carcasses evaluated was due to their independent involvement in the death struggle. The same is likely true for the present study and while described as error within the model should be considered when extrapolating these results to other animals and experimental outcomes.

6.3 Experiment Three

Chroma and b* values were observed to have similar area under ROC curve (AUC) – although chroma had lower specificity and higher sensitivity (Table 5.5). Furthermore, chroma AUC was higher than observed for L* ($P > 0.05$), and significantly higher than a* and hue AUC results ($P < 0.001$). Past research supports these observations. Wulf & Wise (1999) found L*, a* and b* values were each associated to carcass grade (DC vs. nDC) and recommended L* (lightness) because it was less sensitive to blooming time than b*, although b* (yellowness) was more precise in discriminating between carcasses with pH differences. That said, this previous study did not evaluate chroma which is derived from a combination of a* and b* and indicative of relative brightness (AMSA, 2012). Zhang et al. (2018) tested chroma and found that the magnitude of differences between low, intermediate and high pH beef was greater than observed for the other colorimetrics, at 12 h and 24 h *post-mortem*. This was not the case for earlier measurements which were stable, but does comply with the grading window of the present study to suggest chroma as a good discriminator at this juncture. Hughes et al. (2017) also reported DC, light and medium coloured beef to result in colorimetric difference, and variations in lightness (L*) and brightness (chroma and mean pixel intensity) resulted from pH-mediated physical muscle fibre disparities and their 'darkening' influence on light scattering properties. Based on these papers and from the observations of the present study, we can therefore recommend that the chroma threshold of 30.5 is the most suitable for differentiating between carcass grades; as defined, assessed and adhered to by the Australian industry (MLA, 2017).

In practice, it is important to first understand the constraints of this chroma threshold. Fig. 5.4 shows the ROC curves that were used to obtain the optimum thresholds, being the predicted probability of grade at the maximum sum of specificity and sensitivity. This highlights that perfect (1.0) specificity could only be achieved with a cost to sensitivity and therefore, we must expect that any application of an 'optimum' colorimetric threshold would result in instances of false positive and false negative classifications. This is illustrated when observed and chroma threshold estimated carcass grades were compared for the experimental data ($n = 436$), and 6 carcasses were incorrectly graded as DC and 36 as nDC (Fig. 5.5). Consequently, the usefulness of this threshold will depend on the required level of agreement with the benchmark classification.

Other instruments have been used to identify beef carcass grade or pH to differing levels of accuracy and precision. For example, Prieto, Lopez-Campos, Zijlstra, Uttaro, & Aalhus (2014) reported ~ 90% specificity and sensitivity when discrimination between carcass grade classification using Visible-Near Infrared Spectroscopy (Vis-NIRS) technology and intact, bloomed beef samples. Jeremiah, Tong, &

Gibson (1991) reported colorimeter readings to have a higher coefficient of determination (R^2) to muscle pH than grader's colour scores (steers for examples, 0.44 vs. 0.29 respectively). Ponnampalam et al. (2017) reviewed the different pH values (pH range: 5.7-6.3) used to grade beef carcasses and concluded that DC incidence levels are a function of pH threshold selection. Furthermore, Hughes et al. (2014) found beef loins graded as DC using colour chips equivalency to pH classifications was 28% (pH 5.8), 74% (pH 6.0) and 96% (pH 6.2) and was therefore contingent on DC definition. Together, these highlight challenges inherent to all objective appraisals of the somewhat subjective differentiation of DC. A potential response could embed consumer quality expectations into carcass grade definitions through an understanding of the relationship between colorimetrics and meat quality traits.

For the selection of experimental carcasses (n = 30), the estimated change in the meat quality traits associated with chroma values was not significantly different from zero with the exception of striploin and topside pHu (Table 5.6). As pH was used to categorise beef carcasses as DC and colorimetrics can therefore be considered as a proxy for this assessment, the pHu observation is somewhat obvious (Page, Wulf, & Schwotzer, 2001), if not shared in the bolar blade. That said, the fibre type, muscle iron content and oxygen consumption rate/penetration differences between the striploin, topside and the bolar blade may be the basis for this pH disparity (McKenna et al., 2005). When comparing this finding to past research there are inconsistencies. For instance, Wulf, O'Connor, Tatum, & Smith (1997) reported L*, a* and b* values to predict beef shear force and consumer panel tenderness when included within multiple regression equations. Wheeler et al. (2002) demonstrated the palatability index, calculated using L* and b* values, had an efficacy of 70-100% in certifying beef as tender, depending on the certification level required. Furthermore, Beriain, Goni, Indurain, Sarries, & Insausti (2009) found CIE values could predict metmyoglobin percentage and colour stability in aged beef. Together, these studies and others identified the relationship between colour and pH and pH and beef quality traits as fundamental to their results (Grayson et al., 2016). That said, and per the present study, Jeremiah et al. (1991) found colorimeter readings to be poor predictors of beef shear force, albeit better than grader's scores (subjective measures). The range of meat quality traits or pH values represented in this latter study (n = 62) and the present study (n = 30) do suggest sample representation as a likely source for the discrepancy.

7.0 CONCLUSIONS/RECOMMENDATIONS

This study found that carcass grade effects on eating quality traits were not uniformly expressed across the bolar blade, topside and striploin. Only TBARS was influenced by an ageing period and carcass grade interaction. Therefore, it is reasonable to conclude that at least the bolar blade and potentially the forequarter of beef carcasses classified as DC do not reflect the negative attributes of the striploin and topside, and could therefore retain the value of nDC counterparts – particularly for carcasses classified as 'slightly DC' as used in the present study.

The observed variation of glycolysis metabolites and pH between these three cuts over the first 24 h post-mortem was not uniform and therefore provides a biochemical foundation to these organoleptic outcomes. That said, the failure of these traits to stabilise within a 24 h period does suggest that conventional Australian grading practices that occur 14-16 h post-mortem are somewhat premature to capture a metabolically static carcass. This could have implications on DC assessment – albeit noteworthy that the rate of glycolysis was different between individual carcasses.

Nevertheless, when this conventional grading time is adhered to we observed that NIX colorimetrics provide an objective means to differentiate between DC and nDC beef carcasses and as per Australian industry practice. Specifically, a chroma threshold of 30.5 provides the highest total specificity and sensitivity when applied to this task. We identified only limited usefulness for the prediction of other meat quality traits using these same colorimetrics for the bolar blade, striploin and topside cuts. Nonetheless, these outcomes do merit additional research with an increased sample size and scope to confirm their industry potential. Furthermore, the development of alternative thresholds using this same method is encouraged as pH benchmarks for DC and nDC do vary between countries and markets; consequently we acknowledge the somewhat singular application for the proposed chroma threshold to Australia and its comparable beef industries.

8.0 BIBLIOGRAPHY

AMSA. (2012). *Meat color measurement guidelines* (December 2012 ed.). Champaign, Illinois USA: American Meat Science Association (AMSA).

Anonymous. (2005). *Handbook of Australian Meat* (7th ed.). South Brisbane, AUS: AUS-MEAT Ltd.

Bass, P. D., Scanga, J. A., Chapman, P. L., Smith, G. C., Tatum, J. D., & Belk, K. E. (2008). Recovering value from beef carcasses classified as dark cutters by United States Department of Agriculture graders. *Journal of Animal Science, 86*, 1658-1668.

Belew, J. B., Brooks, J. C., McKenna, D. R., & Savell, J. W. (2003). Warner-Bratzler shear evaluations of 40 bovine muscles. *Meat Science, 64*, 507-512.

Bendall, J. R. (1977). Variability in rates of pH fall and of lactate production in the muscles on cooling beef carcasses. *Meat Science, 2*, 91-104.

Bendall, J. R. (1979). Relations between muscle pH and important biochemical parameters during the post-mortem changes in mammalian muscles. *Meat Science, 3*, 143-157.

Beriain, M. J., Goni, M. V., Indurain, G., Sarries, M. V., & Insausti, K. (2009). Predicting *Longissimus dorsi* myoglobin oxidation in aged beef based on early *post mortem* colour measurements on the carcass as a colour stability index. *Meat Science, 81*, 439-445.

Bouton, P. E., Carrol, F. D., Harris, P. V., & Shorthose, W. R. (1973). Influence of pH and fiber contraction state upon factors affecting the tenderness of bovine muscle. *Journal of Food Science, 38*, 404-407.

Bruas-Reignier, F., & Brun-Bellut, J. (1996). Changes affecting the *Longissimus dorsi*, *Triceps brachii caput longum* and *Rectus femoris* muscles of young Friesian bulls during meat ageing. *Meat Science, 43*, 335-344.

Bruce, H. L., Scott, J. R., & Thompson, J. M. (2001). Application of an exponential model to early postmortem bovine muscle pH decline. *Meat Science, 58*, 39-44.

Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D., & Richard, R. I. (2006). Flavour perception of oxidation in beef. *Meat Science, 72*, 303-311.

CIE. (1978). Recommendations on uniform color spaces - color equations, psychometric color terms (pp. 15 (E-11.13.L)). Paris: France: Commission Internationale de l'eclairage (CIE).

Coombs, C. E. O., Holman, B. W. B., Friend, M. A., & Hopkins, D. L. (2017). Long-term red meat preservation using chilled and frozen storage combinations: A review. *Meat Science, 125*, 84-94.

De Brito, G. F., McGrath, S. R., Holman, B. W. B., Friend, M. A., Fowler, S. M., van de Ven, R. J., & Hopkins, D. L. (2016). The effect of forage type on lamb carcass traits, meat quality and sensory traits. *Meat Science, 119*, 95-101.

Dransfield, E. (1994). Optimisation of tenderisation, ageing and tenderness. *Meat Science, 36*, 105-121.

Ferguson, D., & Gerrard, D. (2014). Regulation of post-mortem glycolysis in ruminant muscle. *Animal Production Science*.

Ferguson, D. M., & Warner, R. D. (2008). Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? *Meat Science, 80*, 12-19.

Fischer, C., & Hamm, R. (1979). Biochemical studies of fast glycolysing bovine muscle. *Meat Science, 4*, 41-49.

Forbes, S. M. C., Vaisey, M., Diamant, R., & Cliplef, R. (1974). The relationship between consumer criteria for choosing beef and beef quality. *Canadian Institute of Food Science and Technology Journal, 7*, 130-135.

Grayson, A. L., Shackelford, S. D., King, D. A., McKeith, R. O., Miller, R. K., & Wheeler, T. L. (2016). The effects of degree of dark cutting on tenderness and sensory attributes of beef. *Journal of Animal Science, 94*, 2583-2591.

Holdstock, J., Aalhus, J. L., Uttaro, B., Larsen, I. L., & Bruce, H. L. (2012). *Recovering value from Canada B4 dark cutting beef carcasses*. Paper presented at the 58th International Congress of meat Science and Technology (ICOMST), Montreal, CAN.

Holman, B. W. B., Alvarenga, T. I. R. C., van de Ven, R. J., & Hopkins, D. L. (2015). A comparison of technical replicate (cuts) effect on lamb Warner–Bratzler shear force measurement precision. *Meat Science, 105*, 93-95.

Holman, B. W. B., Collins, D., Kilgannon, A. K., & Hopkins, D. L. (2018). The effect of technical replicate (repeats) on Nix Pro Color Sensor™ measurement precision for meat: A case-study on aged beef colour stability. *Meat Science, 135*, 42-45.

Holman, B. W. B., & Hopkins, D. L. (2019). A comparison of the Nix Colour Sensor Pro™ and HunterLab MiniScan™ colorimetric instruments when assessing aged beef colour stability over 72 h display. *Meat Science, 147*, 162-165.

Holman, B. W. B., Ponnampalam, E. N., van de Ven, R. J., Kerr, M. G., & Hopkins, D. L. (2015). Lamb meat colour values (HunterLab CIE and reflectance) are influenced by aperture size (5mm v. 25mm). *Meat Science, 100*, 202-208.

Holman, B. W. B., van de Ven, R., Mao, Y., Coombs, C. E. O., & Hopkins, D. L. (2017). Using instrumental (CIE and reflectance) measures to predict consumers' acceptance of beef colour. *Meat Science, 127*, 57-62.

Holman, B. W. B., Van de Ven, R. J., Coombs, C. E. O., & Hopkins, D. L. (2017). Effect of beef pre-cooking status (frozen v. thawed) and sample weight on shear force evaluation. *Food Analytical Methods, 10*, 3235-3238.

Honikel, K. O. (2014). Glycolysis. In M. Dikeman & C. Devine (Eds.), *Encyclopedia of Meat Sciences* (2nd ed., Vol. 1, pp. 353-357). London, UK: Academic Press, Elsevier Ltd.

Hopkins, D. L., Clayton, E. H., Lamb, T. A., Van de Ven, R. J., Refshauge, G., Kerr, M. J., Bailes, K., Lewandowski, P., & Ponnampalam, E. N. (2014). The impact of supplementing lambs with algae on growth, meat traits and oxidative status. *Meat Science, 98*, 135-141.

Hopkins, D. L., Toohey, E. S., Warner, R. D., Kerr, M. J., & van de Ven, R. J. (2010). Measuring the shear force of lamb meat cooked from frozen samples: Comparison of two laboratories. *Animal Production Science, 50*, 382-385.

Hughes, J. M., Clarke, F., Purchas, R., & Warner, R. (2017). High pH in beef *longissimus thoracis* reduces muscle fibre transverse shrinkage and light scattering which contributes to the dark colour. *Food Research International, 101*, 228-238.

Hughes, J. M., Kearney, G., & Warner, R. D. (2014). Improving beef meat colour scores at carcass grading. *Animal Production Science, 54*, 422-429.

Hughes, J. M., McPhail, N. G., Kearney, G., Clarke, F., & Warner, R. D. (2015). Beef *longissimus* eating quality increased up to 20 weeks of storage and is unrelated meat colour at carcass grading. *Animal Production Science, 55*, 174-179.

Hulsegge, B., Engel, B., Buist, W., Merkus, G. S. M., & Klont, R. E. (2001). Instrumental colour classification of veal carcasses. *Meat Science, 57*, 191-195.

Jeremiah, L. E., Tong, A. K. W., & Gibson, L. L. (1991). The usefulness of muscle color and pH for segregating beef carcasses into tenderness groups. *Meat Science, 30*, 97-114.

Kim, Y. H., Ma, D., Setyabrata, D., Farouk, M. M., Lonergan, S. M., Huff-Lonergan, E., & Hunt, M. C. (2018). Understanding postmortem biochemical processes and post-harvest aging factors to develop novel smart-aging strategies. *Meat Science, 144*, 74-90.

Kirchofer, K. S., Calkins, C. R., & Gwartney, B. L. (2002). Fiber-type composition of muscles of the beef chuck and round. *Journal of Animal Science, 80*, 2872-2878.

Klont, R. E., Brocks, L., & Eikelenboom, G. (1998). Muscle fibre type and meat quality. *Meat Science, 49*, 219-229.

Lahucky, R., Palanska, O., Mojto, J., Zaujec, K., & Huba, J. (1998). Effect of preslaughter handling on muscle glycogen level and selected meat quality traits in beef. *Meat Science, 50*, 389-393.

Lomiwes, D., Farouk, M. M., Wu, G., & Young, O. A. (2014). The development of meat tenderness is likely to be compartmentalised by ultimate pH. *Meat Science, 96*, 646-651.

Maher, S. C., Mullen, A. M., Keane, M. G., Buckley, D. J., Kerry, J. P., & Moloney, A. P. (2004). Decreasing variation in the eating quality of beef through homogenous pre- and post-slaughter management. *Meat Science, 67*, 33-43.

Mahmood, S., Turchinsky, N., Paradis, F., Dixon, W. T., & Bruce, H. L. (2018). Proteomics of dark cutting *longissimus thoracis* muscle from heifer and steer carcasses. *Meat Science, 137*, 47-57.

Mancini, R. A., & Hunt, M. C. (2005). Current research in meat color. *Meat Science, 71*, 100-121.

McGeehin, B., Sheridan, J. J., & Butler, F. (2001). Factors affecting the pH decline in lamb after slaughter. *Meat Science, 58*, 79-84.

McGilchrist, P., Perovic, J. L., Gardner, G. E., Pethick, D. W., & Jose, C. G. (2014). The incidence of dark cutting in southern Australian beef production systems fluctuates between months. *Animal Production Science, 54*, 1765-1769.

McKenna, D. R., Mies, P. D., Baird, B. E., Pfeiffer, K. D., Ellebracht, J. W., & Savell, J. W. (2005). Biochemical and physical factors affecting discoloration characteristics of 19 bovine muscles. *Meat Science, 70*, 665-682.

MLA. (2017). *Meat standards Australia beef information kit*. www.mla.com.au/msa: Meat & Livestock Australia (MLA) Ltd.

Nix Sensor Ltd. (2018). How do you measure color in your industry? www.nixsensor.com/quality-control-solutions: Nix Sensor Ltd.

Nuss, J. I., & Wolfe, F. H. (1981). Effect of post-mortem storage temperatures on isometric tension, pH, ATP, glycogen and glucose-6-phosphate for selected bovine muscles. *Meat Science, 5*, 201-213.

O'Halloran, G. R., Troy, D. J., & Buckley, D. J. (1997). The relationship between early post-mortem pH and the tenderisation of beef muscles. *Meat Science, 45*, 239-251.

Page, J. K., Wulf, D. M., & Schwotzer, T. R. (2001). A survey of beef muscle color and pH. *Journal of Animal Science, 79*, 678-687.

Pearce, K. L., Rosenvold, K., Andersen, H. J., & Hopkins, D. L. (2011). Water distribution and mobility in meat during the conversion of muscle to meat and ageing and the impacts on fresh meat quality attributes— A review. *Meat Science, 89*, 111-124.

Pethick, D. W., Cummins, L., Gardner, G. E., Knee, B. W., McDowell, M., McIntyre, B. W., Tudor, G., Walker, P. J., & Warner, R. D. (1999). The regulation by nutrition of glycogen level in the muscle of ruminants. *Recent Advances in Animal Nutrition in Australia, 12*, 145-151.

Polkinghorne, R. J., & Thompson, J. M. (2010). Meat standards and grading: A world view. *Meat Science, 86*, 227-235.

Ponnampalam, E. N., Hopkins, D. L., Bruce, H., Li, D., Baldi, G., & Bekhit, A. E. D. (2017). Causes and contributing factors to "dark cutting" meat: Current trends and future directions: A review. *Comprehensive Reviews in Food Science and Food Safety, 16*, 400-430.

Prieto, N., Lopez-Campos, O., Suman, S. P., Rodas-Gonzalez, A., & Aalhus, J. L. (2018). Exploring innovative possibilities of recovering the value of dark-cutting beef in the Canadian grading system. *Meat Science, 137*, 77-84.

Prieto, N., Lopez-Campos, O., Zijlstra, R. T., Uttaro, B., & Aalhus, J. L. (2014). Discrimination of beef dark cutters using visible and near infrared reflectance spectroscopy. *Canadian Journal of Animal Science, 94*, 445-454.

Przybylski, W., Vernin, P., & Monin, G. (1993). Relationship between glycolytic potential and ultimate pH in bovine, porcine and ovine muscles. *Journal of Muscle Foods, 5*, 245-255.

Puolanne, E., & Immonen, K. (2014). Glycogen. In M. Dikeman & C. Devine (Eds.), *Encyclopedia of Meat Sciences* (2nd ed., Vol. 1, pp. 346-352). London, UK: Academic Press, Elsevier Ltd.

R Core Team. (2018). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from www.R-project.org

Robin, X., Turck, N., & Hainard, A. (2011). pROC: an open-source package for R and S+ to analyze and compare ROC curves. *BMC Bioinformatics, 7*, 77.

Savell, J. W., Mueller, S. L., & Baird, B. E. (2005). The chilling of carcasses. *Meat Science, 70*, 449-459.

Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (1995). Relationship between shear force and trained sensory panel tenderness ratings of 10 major muscles from *Bos indicus* and *Bos taurus* cattle. *Journal of Animal Science, 73*, 3333-3340.

Silva, D. R. G., Holman, B. W. B., Kerr, M. J., Morris, S., Ramos, E. M., & Hopkins, D. L. (2018). Effect of homogenisation speed and centrifugation on particle size analysis of beef and the relationship with shear force. *Meat Science, 143*, 219-222.

Talmant, A., & Monin, G. (1986). Activities of metabolic and contractile enzymes in 18 bovine muscles. *Meat Science, 18*, 23-40.

Tarrant, P. V., & Mothersill, C. (1977). Glycolysis and associated changes in beef carcasses. *Journal of the Science of Food and Agriculture, 28*, 739-749.

Wheeler, T. L., Vote, D., Leheska, J. M., Shackelford, S. D., Belk, K. E., Wulf, D. M., Gwartney, B. L., & Koohmaraie, M. (2002). The efficacy of three objective systems for identifying beef cuts that can be guaranteed tender. *Journal of Animal Science, 80*, 33158-33327.

Wu, G., Farouk, M. M., Clerens, S., & Rosenvold, K. (2014). Effect of beef ultimate pH and large structural protein changes with aging on meat tenderness. *Meat Science, 98*, 637-645.

Wulf, D. M., Emnett, R. S., Leheska, J. M., & Moeller, S. J. (2002). Relationship among glycolytic potential, dark cutting (dark, firm, and dry) beef, and cooked beef palatability. *Journal of Animal Science, 80*, 1895-1903.

Wulf, D. M., O'Connor, S. F., Tatum, J. D., & Smith, G. C. (1997). Using objective measures of muscle color to predict beef longissimus tenderness. *Journal of Animal Science, 75*, 684-692.

Wulf, D. M., & Wise, J. W. (1999). Measuring muscle color on beef carcasses using the L*a*b* color space. *Journal of Animal Science, 77*, 2418-2427.

Ylä-Ajos, M., Ruusunen, M., & Puolanne, E. (2006). The significance of the activity of glycogen debranching enzyme in glycolysis in porcine and bovine muscles. *Meat Science, 72*, 532-538.

Zhang, Y., Hopkins, D. L., Zhao, X., van de Ven, R., Mao, Y., Zhu, L., Han, G., & Luo, X. (2018). Characterisation of pH decline and meat color development of beef carcasses during the early postmortem period in a Chinese beef cattle abattoir. *Journal of Integrative Agriculture, 17*, 1691-1695.

9.0 APPENDICES

9.1 List of Publications

Holman BWB, Kerr MJ, Morris S, Hopkins DL (2019) The identification of dark cutting beef carcasses in Australia, using Nix Pro Color Sensor™ colour measures, and their relationship to bolar blade, striploin and topside quality traits. *Meat Science* 148, 50-54

Holman BWB, Hopkins DL (2019) Contrasting the quality traits of aged bolar blade, topside and striploin cuts sourced from dark cutting and control Australian beef carcasses. *Meat Science* (Submitted)

Holman BWB, Zhang Y, Hopkins DL. Relationship between fresh colour (at grading) and colour stability measures for displayed dark or non-dark cutting beef. Proceedings of the '64th International Congress of Meat Science and Technology', 13-17 August 2018, Melbourne: AUS, ref. 6211

9.2 Snapshot

This has been provided as a separate document and also included herein.

SNAPSHOT

CAN ON-SITE BEEF DARK CUTTING EVALUATION (MONITORING) BE IMPROVED AND VALUE-ADDED?

Project Report Reference: 2017-1044 **Date: 16 October 2018**

Project Description

Dark cutting is problematic and in an effort to discourage its prevalence, processors will generally discount and downgrade the value of these carcasses. Their action is based on a preference for bright red beef and as dark cutting beef fails to match this criterion it is instead considered less fresh and of lower quality than normal beef. This fundamental difference results from dark cutting carcasses having insufficient glycogen reserves to drive *post-mortem* acidification which can impact on beef yield and quality characteristics.

In Australia, a trained operator will judge (grade) the exposed *loin surface* of a beef carcass as dark cutting or otherwise within the first 24 hours *post-mortem*. Effectively, a *single marker muscle* is used to grade and discount the entire carcass even though scientific literature reports pH and glycolytic parameter differences within-carcass and between muscles. As a consequence, there could be an opportunity to recapture these otherwise discount cuts from dark cutting carcasses and instead direct them towards more premium product types.

Project Content

This project explored glycolytic and sensory quality parameter variation within carcasses graded as dark cutting and compared these to normal carcasses. The cuts evaluated were the bolar blade, striploin and topside. Instrumental colour measurements made at grading were assessed in terms of their capacity to identify dark cutting and predict other eating quality traits for these same cuts.

Project Outcome

A comparison of three beef cuts from dark cutting and normal carcasses found that at least the bolar blade and potentially the forequarter of beef carcasses classified as dark cutting did not reflect the negative attributes of the striploin and topside. This outcome was supported by the differences in glycolytic derivate and pH decline differences observed between these same beef cuts.

Modelling pH and glycolytic parameter (incl. glucose, glycogen, lactate, and glucose-6-phosphate) trends in these same three cuts found their levels continued to change even at 24 h post slaughter. This suggests that conventional grading (12-16 h post-mortem) may be premature and misrepresent the carcass as dark cutting – previous research projects support this observation.

Based on the colour of the exposed *loin* surface between the 12-13th rib, carcasses measured at grading, we found that a chroma value equal to or greater than 30.5 was most indicative of the carcass being a dark cutter. This outcome conditional on a permissible degree of error, that pH > 5.7 is indicative of dark cutting, and colour measurements are made using a Nix Pro Color Sensor™.

Benefit for Industry
Applying these findings, it is reasonable to conclude that components of a dark cutting beef carcass could be *salvaged* to regain a proportion of its undiscounted value. This could mitigate some of the associated economic and environmental impacts incurred from maybe not-so-inferior meat products.

If adopted, the chroma threshold for dark cutting identification could provide an objective alternative to current subjective methods of colour assessment. Furthermore, this approach could avoid many risks associated with inserting a glass pH probe into what is ultimately an edible product (i.e. breakages, etc.)

USEFUL RESOURCES

// Improving Beef Colour at Grading – Final Report | **2013-3005**
// Causes and Contributing Factors to Dark Cutting: Current Trends and Future Directions | **2014-1060**